Evidence for a Pheromone in the Marine Periwinkle, *Littorina littorea* Linnaeus

BY

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(1 Text figure)

INTRODUCTION

A PHEROMONE IS A SUBSTANCE released by an animal which, if sensed by a second animal of the same species, causes a change in the probability pattern of the receiving animal's behavior. For an excellent review of chemical communication in animals the reader is referred to an article by WILSON, 1970.

MATERIALS AND METHODS

Specimens of *Littorina littorea* Linnaeus, 1758, were collected at the rocky shore of Nahant, Massachusetts. Fresh sea water was also collected. Experiments were performed within 3 days of the collection day.

Our first attempts to set up a situation in which a specimen of *Littorina littorea* could "choose" to move toward another member of its species were failures due to the fact that the snail usually would not move at all. Then we took advantage of the fact that periwinkles are negatively geotropic in the dark (KANDA, 1916). We placed the snails on plates of glass to which they adhered, set the glass at a vertical angle in an aquarium and covered the aquarium with a foil-covered, light-proof cardboard box. Under these conditions most snails moved upward.

Five-gallon capacity aquaria were used for the experiments. Two glass test tubes (192×36 mm) were attached to a glass plate with waterproof electrical tape (Figure 1). A plastic platform taped 30 mm below the test tube openings served as a holder for the experimental animals. The glass plate was inserted into the aquarium and set at an angle of approximately 34° from the vertical.

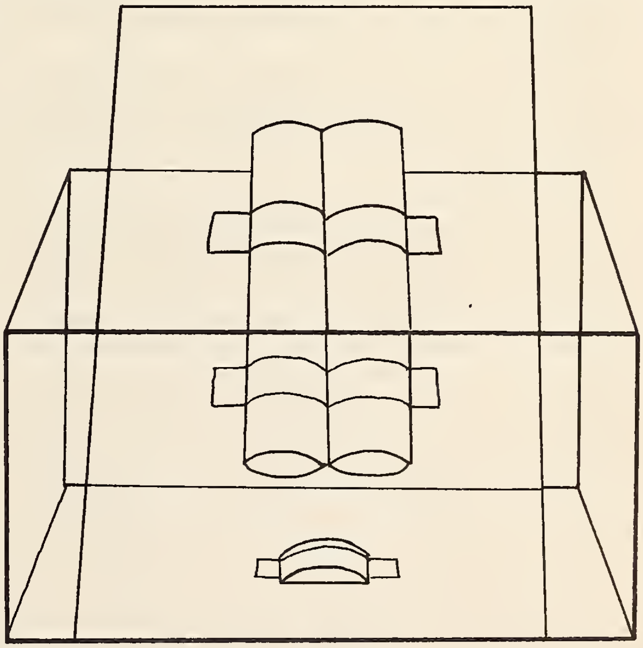

Figure 1

Apparatus for the demonstration of a pheromone in *Littorina littorea*

Attracting snail is placed at top of right tube, test snail is placed on starting platform

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The tubes and aquarium were filled with seawater. Usually an experiment was run with a series of 10 test snails. A snail was placed on the platform and allowed to move up the plate or into the left or right tube. Fifty-six percent of all snails run entered a test tube. All the data presented in this report are for snails which entered one tube or the other. At the beginning of the series the water temperature was 7° C, at the end 14 ± 2° C. All experiments were performed in darkness.

EXPERIMENTS AND RESULTS

Experiment 1 was intended to show if *Littorina littorea* demonstrated any preferred direction of movement with no guiding stimulus present. The tubes were filled with fresh seawater, a periwinkle placed on the platform by hand, and the aquarium covered. After 10 minutes the box was removed and the position of the snail recorded. The snail was then marked, measured, and its sex identified (Linke, 1933; Fretter & Graham, 1962). A new snail was placed on the platform and the experiment repeated. Whenever a snail entered a tube, the glass plate and tubes were replaced with fresh ones in order to eliminate any possibility of build-up of substances within the tubes.

The results showed that *Littorina littorea* has a strong tendency to move to the left side (Table 1). Of the females 84% (39 out of 46) and of the males 75% (24 out of 32) entered the left tube.

Since periwinkles naturally tended to move to the left, we set up Experiment 2 in which an "attracting" snail was placed into the right tube and allowed to adhere. Both tubes were filled with seawater and rubber stoppers prevented convection currents from mixing the water in the tubes with that in the rest of the aquarium. The rationale was to allow any substance emitted by the "attracting" snail to become concentrated within the right tube. After one hour the stoppers were removed and the experiment conducted as before. Each time a snail entered a tube it was carefully removed by prying it loose with a spatula. If it went into the left tube, the water was blown out with rubber tubing and the test tube then refilled.

The results of this experiment showed that when a snail was present in the right tube other snails exhibited a strong tendency to enter that tube (Table 1). Of the females 62% (31 out of 50) and of the males 66% (26 out of 39) entered the right tube. To demonstrate the statistical significance of the difference between experiments 1 and 2 we assumed that there was no expected difference (the null hypothesis) and calculated the chi-square value on this assumption (Table 1). The probability that these results occurred by chance alone is less than 0.005.

Experiment 3 was designed to eliminate the possibility that the test snail was being attracted into the right tube by other than chemical stimuli. A specimen of *Littorina littorea* was inserted into the right tube and left for 5 hours. Both tubes were stoppered. The snail was then removed from the tube to insure that only the pheromone, if present, would be attracting other snails. This experiment was then performed like Experiment 2.

The results again showed that snails preferentially entered the right tube (Table 1). Comparing these results with those of Experiment 1, using the null hypothesis, shows that this distribution of data would occur by chance with a probability of less than 0.005.

Table 1

Preferential Entry of Left or Right Tube

	Number of snails entering	
	left tube	right tube
Experiment 1	63	15
Experiment 2	32	57
Experiment 3	3	11

for Experiments 1 and 2, $X^2 = 32.2$, $p < 0.005$
for Experiments 1 and 3, $X^2 = 17.8$, $p < 0.005$

DISCUSSION

Even a cursory glance at Table 1 gives the impression that snails are attracted into the right tube in Experiments 2 and 3. When these results are compared, using the null hypothesis, with the results of Experiment 1, the statistical significance of the results is even clearer. In both cases these results could be expected to occur by chance only 5 times in 1000 trials.

The simplest hypothesis which explains these results is that *Littorina littorea* emits a substance (or substances) which attracts other snails.

A total of 321 specimens of *Littorina littorea* were tested. It was demonstrated that periwinkles have a strong tendency to move up to the left in the dark. It is of interest to compare this observation with that made by Hayes (1929) on *L. littorea* moving on a horizontal surface: "It may be said that the individuals appeared to be totally indifferent as to which way they turned ... The fact that the shells are dextral appears to have no effect in determining such activities." Either an unequal distribution of weight or the snails' inherent dextrality might explain why, in Experiment 1, 4 times as many snail moved to the left as to the right.

We found no significant difference in the ability of males or females placed in the right tube to attract either males or females. We did note, however, a tendency for a higher percentage of snails to enter the right tube (in either Experiment 2 or 3) as the season changed from early March to early May. It is of interest that spawning takes place in March, April, and May, in this species (LINKE, 1933; FRETTER & GRAHAM, 1952; WILLIAMS, 1964; 1970). After this period the reproductive organs of males and females are reduced to a minimum size and develop again the following spring.

Snails ranging from 7.8 mm to 13 mm in height of shell never entered a right tube, seldom a left tube, instead usually moved to the right or the left of the glass plate. It has been shown that only rarely do specimens below 11 to 12 mm in shell height attain sexual maturity (WILLIAMS, 1970). Snails ranging from 13 to 26 mm in shell height entered the right tube in Experiment 2 and Experiment 3. These facts along with the observation that a higher percentage of animals moved into the right tubes as the mating season progressed indicate that a sex pheromone may be involved.

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